LA-UR-20-23926
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Title: Biogenic uranium isotope fractionation

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Intended for: Share with collaborator

Issued: 2020-05-28

Biogenic uranium isotope fractionation

Team presentation

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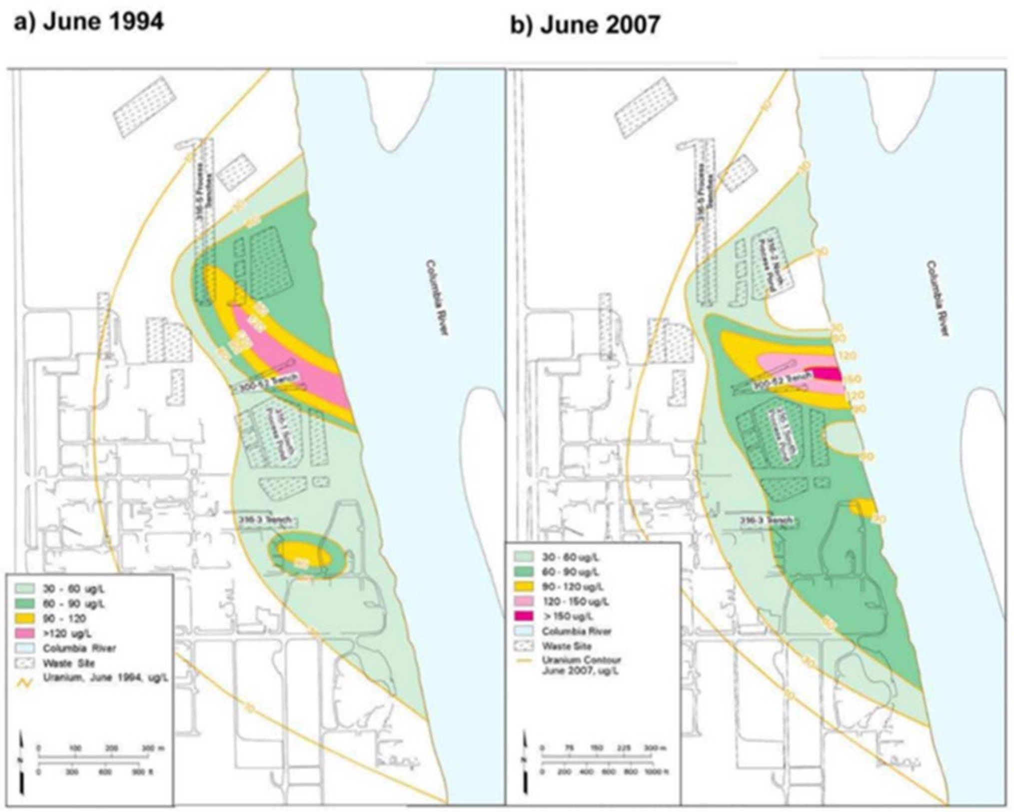
Outline

- Background and relevance
- Project objectives
- Tasks and timeline
- Current Activities
  - Task 1: Controlled culture growth
  - Task 2: Enzymatic reduction of uranium oxides
  - Task 3: Cellular location of uranium reduction and precipitation
- Future work
- Achievements

Uranium biogeochemistry

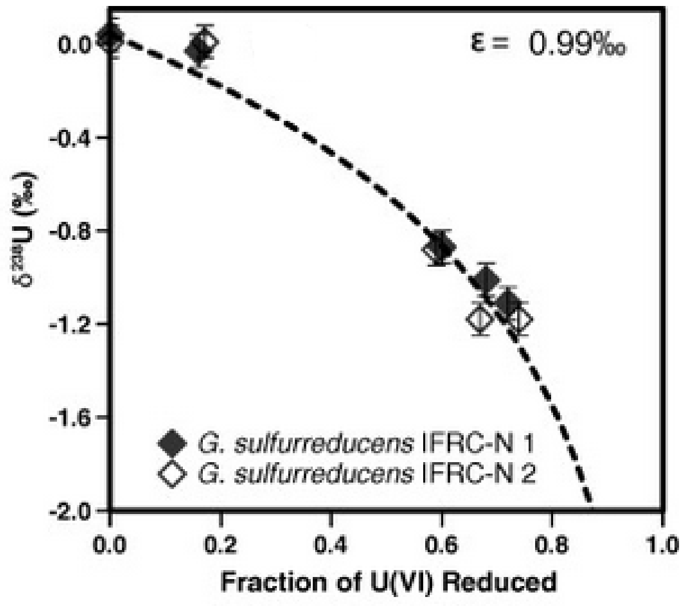
- Uranium chemistry controls how U ore deposits form, how to clean up U contamination, and how U is distributed in the environment
- U is redox-active
  - U(VI)- soluble and mobile in water systems
  - U(IV)- relatively insoluble and primarily in sediment systems
- Common method to remove U from solution is microbial U(VI) reduction
- However, uranium concentrations impacted by multiple chemical and physical processes



Yabusaki et al., 2008

Uranium isotopes

- Uranium isotopes ($^{238}U/^{235}U$) can provide a more direct indicator of U(VI) reduction
- Not strongly affected by adsorption or physical processes
- Microbial U(VI) reduction- preferential reduction of ^{238}U (Basu et al., 2014; Stylo et al., 2015)
  - Less ^{238}U remaining in U(VI) solution
- Abiotic reduction produces a large range of isotopic fractionation (Brown et al., 2018; Stylo et al., 2015)
- What are the processes and mechanisms controlling isotope fractionation?



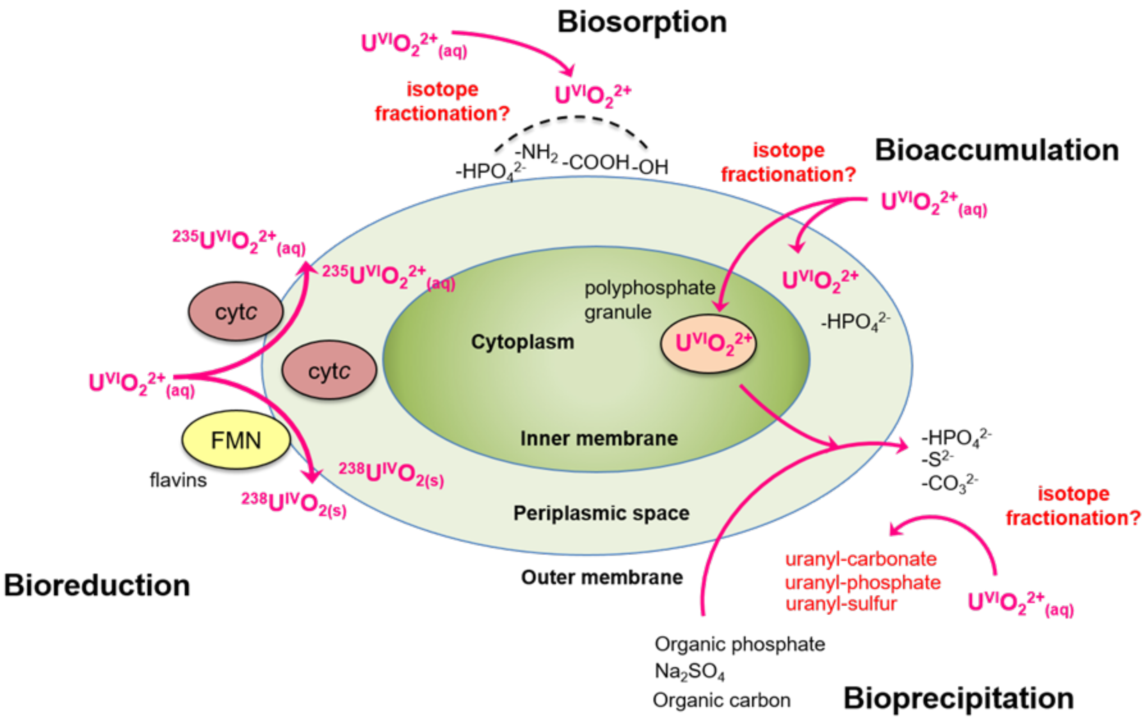
Basu et al., 2014

What affects fractionation?

- Isotopic fractionation defined as:

  $\varepsilon = {}^{238}U/{}^{235}U_{U(IV)\ product} \;/\; {}^{238}U/{}^{235}U_{U(VI)\ reactant}$

- Aqueous chemistry impacts isotope fractionation during abiotic experiments
- May be due to U(VI) speciation or reduction rate
- U(VI) reduction to U(V) and then disproportionation to U(IV) and U(VI)?
- Biosorption, bioaccumulation, and bioreduction mechanisms?
- What are the primary factors controlling the magnitude and direction of isotope fractionation during U(VI) reduction?
- Can we reliably apply $^{238}U/^{235}U$ to track and quantify U(VI) reduction in natural environments?



Project objectives

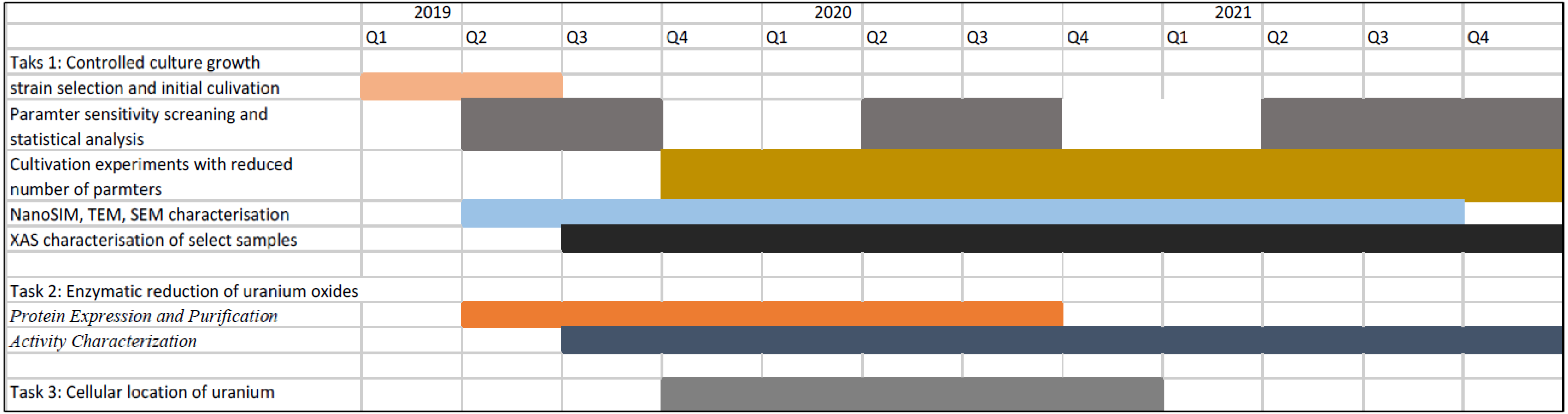
- The underlying goal is to determine the mechanistic driver(s) of U fractionation, probing processes from the initial interaction between the cell and soluble U to the accumulation of U mineral precipitates near or within the cell.
- We will focus on the characterization of three aspects of uranyl bioreduction that likely control U isotope fractionation:
  - **1.** kinetic controls that dictate U adsorption, sequestration, and/or uptake and its subsequent reduction;
  - **2.** cellular processes that support the electron transport pathways and enzymatic reduction of uranium;
  - **3.** characterization and mapping of the cellular location of U reduction and precipitation.

Impact of research

- Determining the primary mechanisms of U isotope fractionation would establish LANL as a leader in environmental isotope measurements
- Develop our capabilities for tracing environmental biogeochemical reactions
- Gain more recognition for emerging isotope measurements and applications

Project timeline

Schedule and Milestones



FY-19 activities

- Task 1: Controlled culture growth
  - Strain selection and initial cultivation
  - Parameter sensitivity screening and statistical analysis
  - Cultivation experiments with reduced number of parameters
  - Nano SIM, TEM, SEM characterization
  - XAS characterization of select samples
- Task 2: Enzymatic reduction of uranium oxides
  - Protein Expression and Purification
  - Activity Characterization
- Task 3: Cellular location of uranium
  - Uranium uptake
    - Cellular Sorption
    - Intracellular uptake

Strain selection and initial cultivation

- Currently, growing Shewanella oneidensis and Pelosinus strain UFO1
  - Both capable of U(VI) reduction
  - Shewanella- facultative anaerobe, gram (-)
  - Pelosinus- strict anaerobe, gram (+)
- Any differences in isotope fractionation due to different microbial mechanisms?
- How does aqueous chemistry affect isotope fractionation during reduction by these microbes?
- Could microbial uptake impact observed isotope fractionation?

Parameter sensitivity screening

- **Abiotic U(VI) reduction experiments**
  - Abiotic experiments eliminate some of the complexity of microbial experiments
  - These experiments allow us to screen for what parameters strongly affect isotope fractionation during U(VI) reduction
  - How does U(VI) speciation, solution chemistry, and reduction rate impact isotope fractionation?

Methods

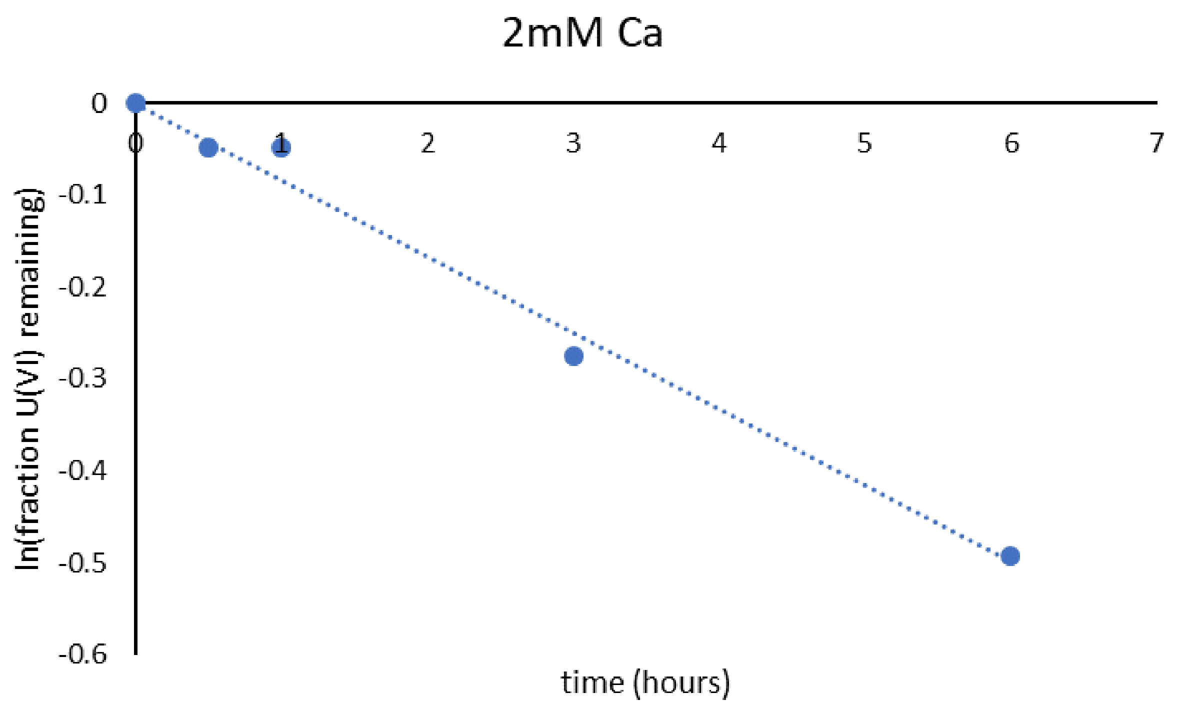
- Performed batch experiments in an anaerobic chamber where reductant was added to U solutions
- Reductants: FeS and Na_2S with quartz
- Varied chemistry
  - pH (6.5 and 7)
  - bicarbonate (6 and 2mM)
  - Ca (0, 1, and 2mM)
  - Mg (0 and 10mM)
  - MOPS pH buffer (10 and 70mM)
- Calculated U(VI) speciation and adsorption coefficients (K_D) using CrunchTope

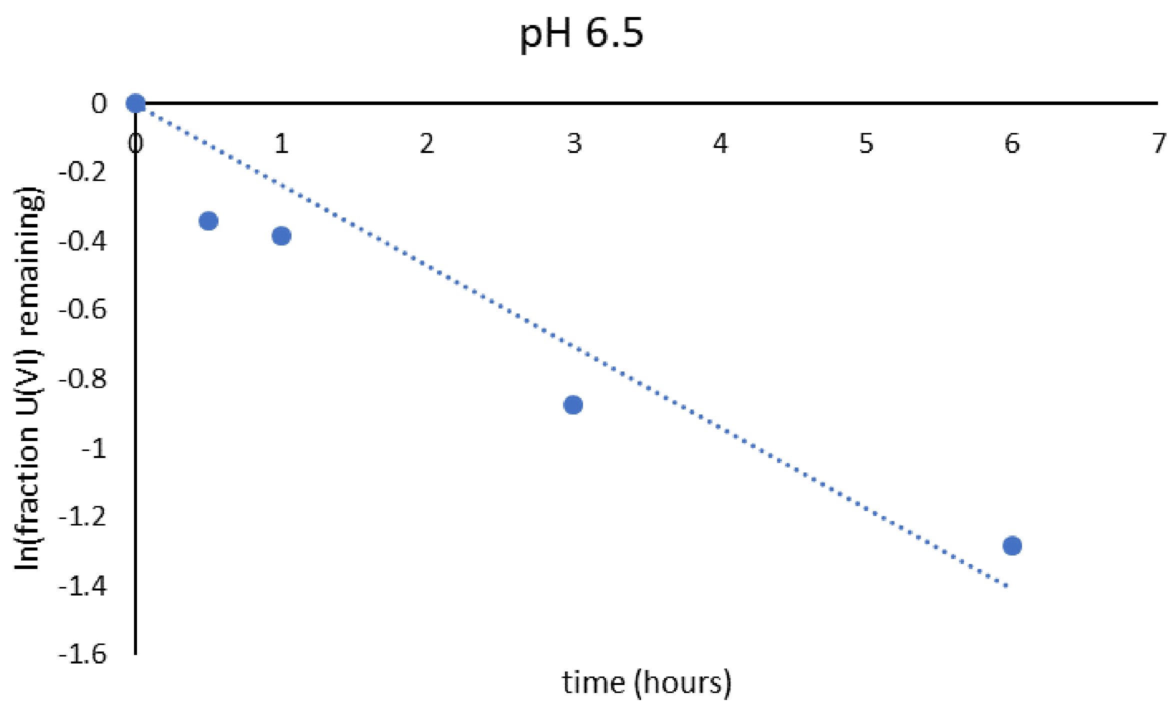
Experiment	mM Ca	mM Mg	mM HCO3	mM MOPS	pH	reductant
1	1	0	6	70	7	FeS
2	1	0	6	70	7	FeS
3	0	0	6	70	7	FeS
4	0	0	6	70	7	FeS
5	2	0	6	70	7	FeS
6	2	0	6	70	7	FeS
7	2	0	6	70	7	FeS
8	0	10	6	70	7	FeS
9	1	0	6	10	7	FeS
10	1	0	2	70	7	FeS
11	1	0	6	70	6.5	FeS
12	1	0	6	70	6.5	FeS
13	1	0	6	70	7	HS
14	1	0	2	70	7	HS

Isotope methods

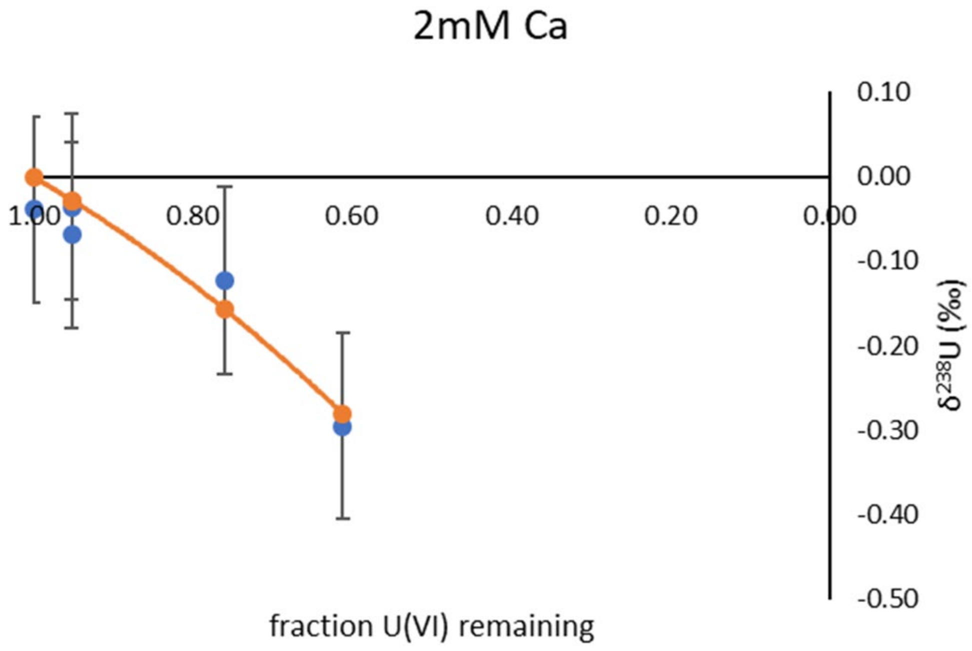
- Collected samples over time as U(VI) was reduced
- Samples filtered to remove reductant and U(IV)
- Analyzed remaining U(VI)
- Added ^{233}U-^{236}U double spike to U(VI) samples to account for mass bias
- Measured U(VI) concentrations and $\delta^{238}U$ on a multi-collector ICPMS (MC-ICPMS) at University of Illinois
- $\delta^{238}U = ({}^{238}U/{}^{235}U_{sample} / {}^{238}U/{}^{235}U_{112A\ std} - 1) * 1000‰$

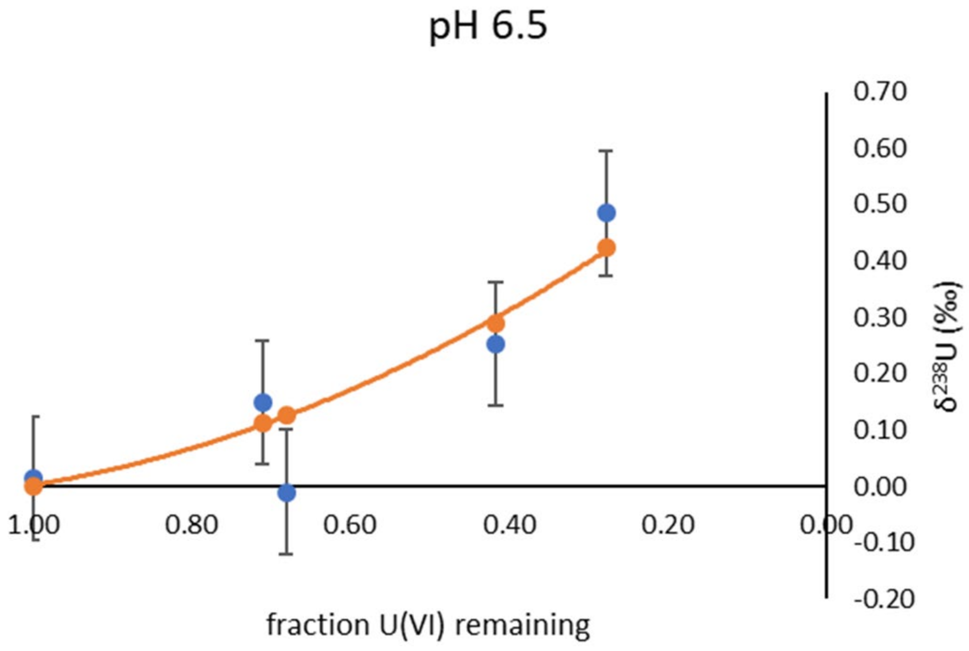
Concentration data



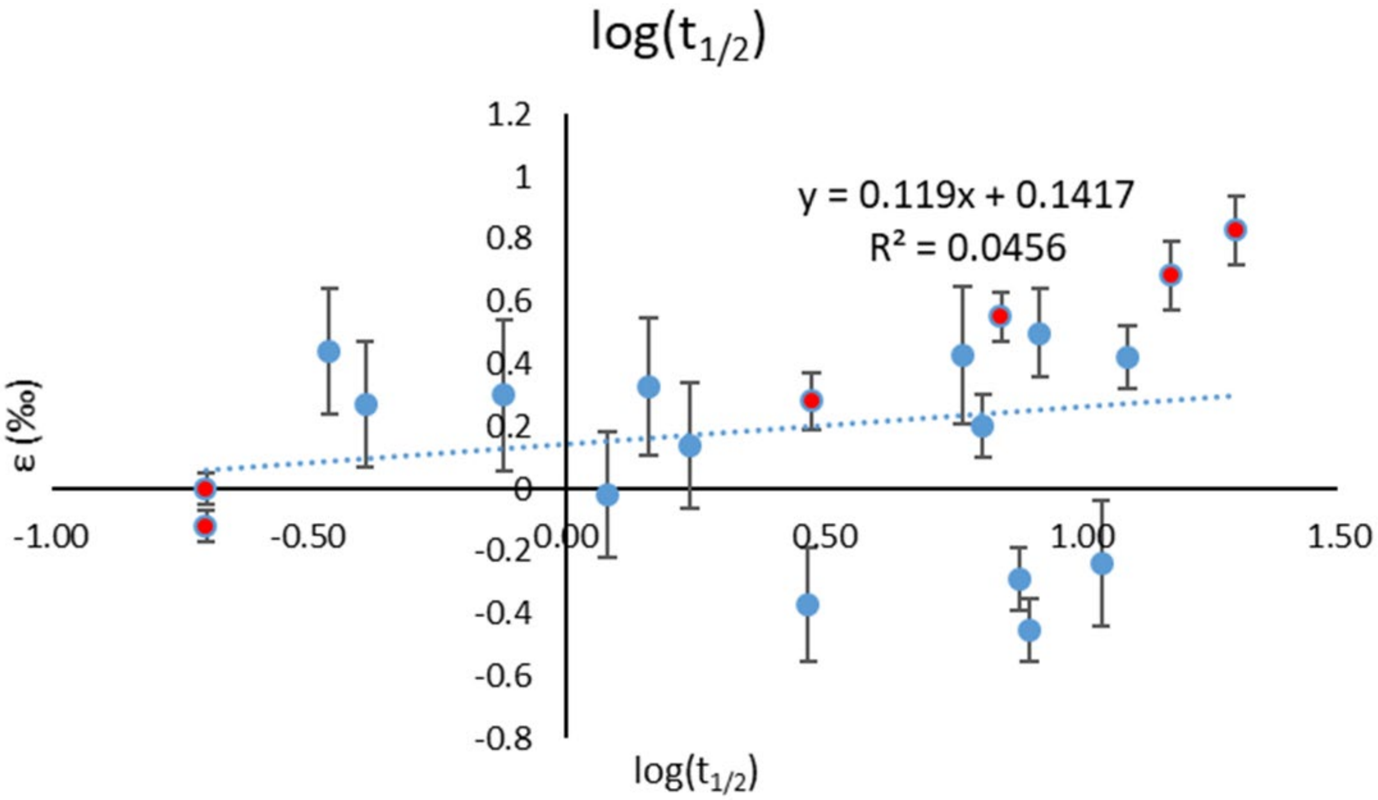


Isotope Data

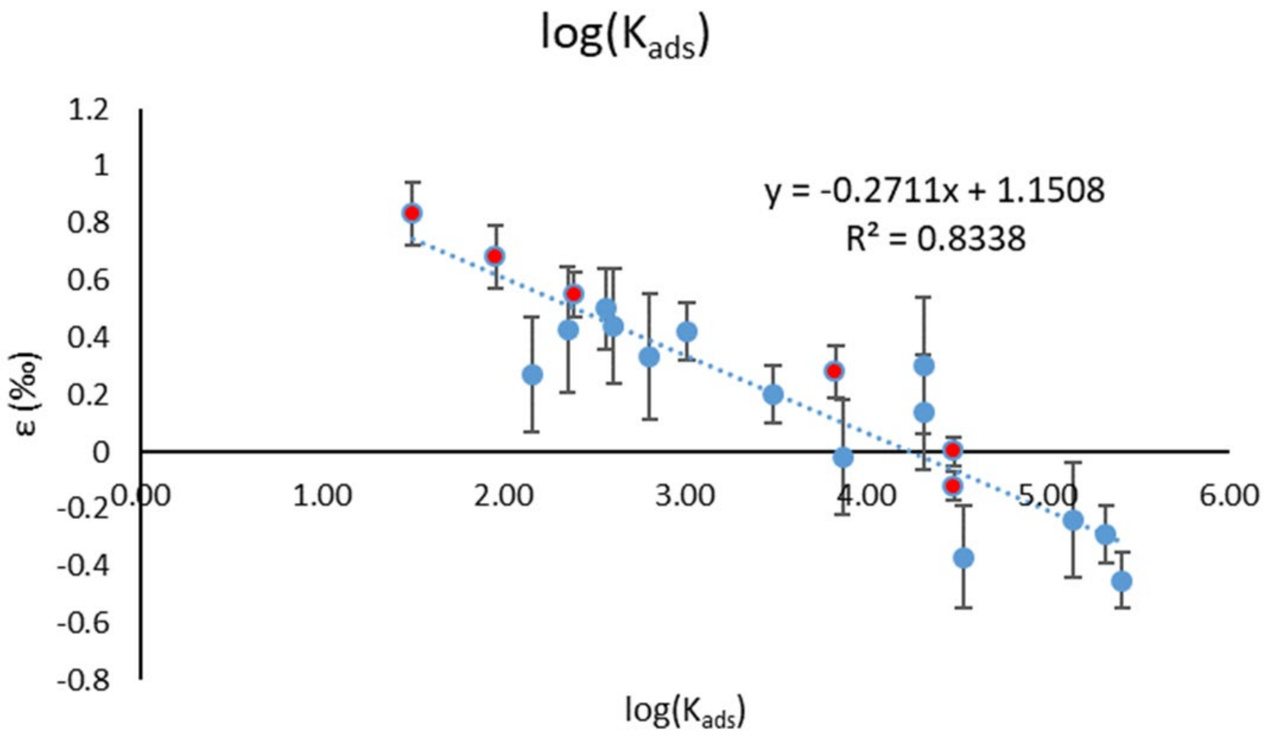




Fractionation mechanisms?



Fractionation mechanisms?



Parameter sensitivity discussion

- Adsorption coefficient much more strongly correlated with ε than U(VI) speciation or reduction rate
- Aqueous chemistry can influence U isotope fractionation through adsorption and then reduction of U(VI)
- U(VI) adsorption induces an isotopic fractionation of ~0.2‰ (adsorbed U(VI) enriched in ^{235}U) (Jemison et al., 2016)
- Fractionation remains intact when K_D is high, but with low K_D, ε is dominated by U(VI) reduction (^{238}U preferentially reduced)

Parameter sensitivity discussion

- With high K_D, most U(VI) that is adsorbed is reduced
- With low K_D, more U(VI) can desorb and communicate with the aqueous U(VI) pool
- What about microbial U(VI) reduction?
  - Need to test how aqueous chemistry impacts U isotope fractionation

Enzymatic reduction of uranium oxides

- Chris will update
  - **Protein Expression and Purification**
  - **Activity Characterization**

FY-19 Accomplishments

- Jemison, N.; Reimus, P.; Harris, R.; Boukhalfa, H.; Clay, J.; Chamberlain, K. Reduction and potential remediation of U(VI) by dithionite at an in-situ recovery mine: insights gained by $\delta^{238}U$. Applied Geochemistry. Submitted. (LA-UR-19-27182)
- Jemison, N.; Boukhalfa, H.; Marti-Arbona, R.; Yeager, C.; Ning, X. Mechanisms of Uranium Isotope Fractionation. Poster, Goldschmidt 2019. (LA-UR-19-22703)

Current activities

- Task 1: Controlled culture growth
  - Strain selection and initial cultivation
  - Parameter sensitivity screening and statistical analysis
  - Cultivation experiments with reduced number of parameters
  - **Nano SIM, TEM, SEM characterization**
  - XAS characterization of select samples
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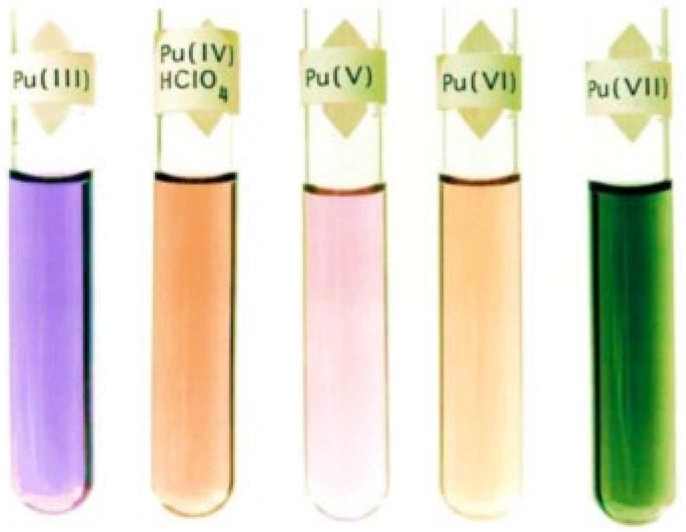
Microbial Pu reduction

- Will soon reduce Pu(VI) to Pu(V) and Pu(IV) using Shewanella and Pelosinus
- Allows us to see fractionation for each electron step
  - U(VI) reduction does not produce significant amounts of stable U(V) species
- First study on Pu isotope fractionation during natural reduction processes



External Collaborators

- Tom Johnson (University of Illinois- Urbana-Champaign)
- John Cliff (Pacific Northwest National Laboratory)
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